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Journal of Controlled Release 71 (2001) 53–69

journal of controlled release

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Effects of emulsifiers on the controlled release of paclitaxel (Taxol®) from nanospheres of biodegradable polymers

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Received 22 September 2000; accepted 24 November 2000

Abstract

Paclitaxel (Taxol®) is an antineoplastic drug effective for various cancers especially ovarian and breast cancer. Due to its high hydrophobicity, however, an adjuvant such as Cremophor EL has to be used in its clinical administration, which causes serious side-effects. Nanospheres of biodegradable polymers could be an ideal solution. This study investigates the effects of various emulsifiers on the physical/chemical properties and release kinetics of paclitaxel loaded nanospheres fabricated by the solvent extraction/evaporation technique. It is shown that phospholipids could be a novel type of emulsifiers. The nanospheres manufactured with various emulsifiers were characterized by laser light scattering for their size and size distribution; scanning electron microscopy (SEM) and atomic force microscopy (AFM) for their surface morphology; zeta potential analyser for their surface charge; and, most importantly, X-ray photoelectron spectroscopy (XPS) for their surface chemistry. The encapsulation efficiency and in vitro release profile were measured by high performance liquid chromatography (HPLC). It is found that dipalmitoyl-phosphatidylcholine (DPPC) can provide more complete coating on the surface of the products which thus results in a higher emulsifying efficiency compared with polyvinyl alcohol (PVA). Our result shows that the chain length and unsaturation of the lipids have a significant influence on the emulsifying efficiency. Phospholipids with short and saturated chains have excellent emulsifying effects. © 2001 Published by Elsevier Science B.V.

Keywords: Lipid chain length; Lipid chain unsaturation; Natural emulsifiers; Paclitaxel; Single emulsion

1. Introduction

Paclitaxel (Taxol®) is a diterpenoid extracted from the bark of a rare, slowly growing Pacific yew or Western yew tree (Taxus brevifolia). Its anti-tumor activity was detected in 1967 in the US National Cancer Institute (NCI) screening of cytotoxic agents from natural products and was then found later to be a novel, promising antineoplastic drug of special effect for breast and ovarian cancers [1]. Its therapeutic efficacy also includes head and neck cancer, small cell lung cancer, colon cancer, multiple myeloma, melanoma, and Kaposi's sarcoma. It was approved by US Food and Drug Administration (FDA) for ovarian cancer in 1992, for advanced breast cancer in 1994 and for early stage breast

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0168-3659/01/$ – see front matter
PII: S0168-3659(00)00364-3

cancer in October 1999. The action mechanism of paclitaxel has been intensively investigated and the results have suggested that paclitaxel acts to inhibit mitosis in tumour cells by binding to microtubules. Microtubules are made up of tubulins and involved in various cellular functions, such as cell movement, nutrition ingestion, cell shape control, sensory transduction and spindle formation during cell division. Paclitaxel aids polymerization of tubulin dimers to form microtubules and thus stabilizes the microtubules. The microtubules formed due to paclitaxel action are stable and thus dysfunctional, leading to cell death [2–4].

There are two limitations for clinical application of paclitaxel. One is its availability. Although extraction of paclitaxel in large scale has increased yields to 0.04%, four trees have to be sacrificed to produce 2 g of the drug for the chemotherapy of one patient. This is not affordable from an environmental point of view. Although having been achieved, full synthesis is not practical, as more than 200 steps are needed and the price is thus too high. An appropriate solution seems to be a semi-synthesis, which extracts paclitaxel from needles and twigs of more abundant English yew trees, or Chinese red bean yew trees. Another limitation is its difficulty in clinical administration. Paclitaxel is highly hydrophobic (water insoluble, with water solubility $\leq$0.5 mg/l). An adjuvant consisting of Cremophor EL (polyoxyethylated castor oil) and dehydrated alcohol has to be used in current clinical administration of paclitaxel, which causes serious side-effects. It has been believed that side-effects caused by Cremophor EL include hypersensitivity reactions, nephrotoxicity and neurotoxicity. It was also reported that Cremophor EL has influence on the functions of endothelial and vascular muscle and causes vasodilation, laboured breathing, lethargy, and hypotension. In application, it causes leaching of diethylhexylphthalate (DEHP) from plasticized polyvinyl chloride (PVC) containers and intravenous infusion line [5–10].

Due to the limitation of paclitaxel in its clinical administration, its dosage and infusion period have thus been restricted. Best clinical effects have not been achieved yet. Alternative dosage forms are thus necessary to overcome the problem caused by Cremophor EL. Such alternative dosages may include liposomes [11–13], mixed micelles [14], parenteral emulsions [15,16], and cyclodextrin complexes [17]. Among them, some dosage forms can dissolve sufficient quantities of paclitaxel and have shown improved anti-tumor effects in animal models. However, problems such as the in vivo instability of liposomes and dose-limiting toxicity due to the vehicles have also been noticed [15,17].

The use of biodegradable polymeric micro/nanospheres for controlled delivery of anticancer agents has advantages in enhancing therapeutic efficacy and reducing systemic side-effects. Certain satisfactory results in clinical trials have been obtained [18,19]. In addition, it has been reported that nanospheres show significant advantages over microspheres [20–22]. Nanospheres enable intravenous injection as well as intramuscular and subcutaneous administration by minimizing possible irritant reactions.

Our group has investigated the feasibility to apply nanospheres of biodegradable polymers as an alternative administration system for paclitaxel. The biodegradable polymers used in this research include poly(D,L-lactic-co-glycolic acid) (PLGA) (lactic/glycolic ratio 50/50 or 75/25) and poly(D,L-lactic acid) (PLA), all FDA approved. The modified solvent extraction/evaporation technique, or the single emulsion technique, was applied in this study to prepare nanospheres under various fabrication conditions. The first step of this technique is to formulate an emulsion. The oil phase formed by dissolving the drug and the polymer in an organic solvent is dispersed in the aqueous phase. This is followed by evaporation of the organic solvent. Since the oil drops in the emulsion are easy to aggregate in the aqueous phase, the emulsifier, which is supposed to prevent the aggregation of oil phase, plays an important role in the process.

Emulsifiers have a wide variety of applications. For example, in foods, they promote the suspension of one liquid in another as in the mixture of oil and water in margarine, shortening, ice cream, and salad dressing. Emulsifiers are also used in the preparation of cosmetics, lotions, and certain pharmaceuticals, where they serve much the same purpose as in foods, i.e. they prevent separation of ingredients and extend storage life. Macromolecular emulsifiers, which include gelatin [23] and PVA (polyvinyl alcohol) [24], are widely used in the fabrication of polymeric micro/nanospheres due to their high viscosity in the

aqueous solution and strong adsorption around the emulsion drops. However, these macromolecules are not easily washed out of the micro/nanospheres [25]. As a result, they may cause troubles in purification of products and thus influence the properties of the formed micro/nanospheres. Among various natural emulsifiers, phospholipids have been widely used as wetting and emulsifying agents and for other purposes in industry. The products in which phospholipids are used as emulsifiers include animal feeds, baking products and mixes, chocolate, cosmetics and soaps, dyes, insecticides, paints, and plastics. However, their application as emulsifiers in the solvent extraction/evaporation technique to fabricate polymeric micro/nanospheres as a drug delivery system has rarely been reported. Only a few publications suggested that the use of 1,2-dipalmitoylphosphatidylcholine (DPPC) as an additive may be able to improve the performance of the produced PLGA microspheres in blood flow [26], enhance the pulmonary absorption of peptides and proteins [27] and reduce phagocytic uptake of the microspheres [28]. All these facts hint at the potential application of phospholipids as natural emulsifiers in the double or single emulsion technique to fabricate polymeric micro/nanospheres.

It is therefore the emphasis of this paper to recognize and characterize this new type of emulsifier. We shall prove that compared with the traditional chemical emulsifiers such as PVA, phospholipids are of higher efficiency in emulsion stabilization and can result in polymeric micro/nanospheres of better physical/chemical properties. Characterization of paclitaxel-loaded polymeric nanospheres fabricated with various emulsifiers is conducted by laser light scattering for their size and size distribution, scanning electron microscopy (SEM) and atomic force microscopy (AFM) for their surface morphology and X-ray photoelectron spectroscopy (XPS) for the chemical structure of their surface. The drug encapsulation efficiency and the in vitro release kinetics are measured by high performance liquid chromatography (HPLC). Except for recognition of phospholipids as effective emulsifiers, another feature of this paper is its use of atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) to characterize the prepared nanospheres and to manifest the emulsifier's role in the formation of the micro/nanospheres. It is found that compared with traditional chemical emulsifiers such as polyvinyl alcohol (PVA), phospholipids can provide a more complete coating on the surface of the products, which thus results in a higher emulsifying efficiency. Phospholipids of various chain length and chain unsaturation such as 1,2-didecanoylcphosphatidylcholine (DDPC), 1,2-dipalmitoylphosphatidylcholine (DPPC), 1,2-distearoylphosphatidylcholine (DSPC) and 1,2-dioleoylphosphatidylcholine (DOPC) are investigated for their emulsifying effects in the single emulsion process in fabrication of paclitaxel loaded nanospheres. The result shows that the length and unsaturation of the lipid chains have significant influence on the emulsifying efficiency. Phospholipids with short and saturated chains such as DDPC can have excellent emulsifying effects.

2. Materials and methods

2.1. Materials

Paclitaxel (Taxol®) is the product of Dabur India (India) or Hande Biotechnology (China). Polymers such as poly(D,L-lactic-co-glycolic acid) (PLGA) with L:G molar ratio of 75:25 and 50:50 (MW= 90,000–120,000) and poly(lactic acid) (PLA), chemicals such as polyvinyl alcohol (PVA) (MW= 30,000–70,000) and methylene chloride (DCM), and various lipids such as 1,2-didecanoylcphosphatidylcholine (DDPC), 1,2-dipalmitoylphosphatidylcholine (DPPC), 1,2-distearoylphosphatidylcholine (DSPC) and 1,2-dioleoylphosphatidylcholine (DOPC) were all purchased from Sigma (St. Louis, MO).

2.2. Methods

2.2.1. Preparation of polymeric nanospheres

Paclitaxel and 200 mg of PLGA were dissolved in 16 ml of methylene chloride (DCM). The formed solution was subsequently added to 250 ml of distilled water. The resulting emulsion was sonicated with an energy output of 50 W in a pulse mode for 90 s. The oil-in-water emulsion was then stirred overnight at room temperature with the magnetic

stirrer to evaporate methylene chloride. The produced nanospheres were collected by centrifugation (12,000 rpm, 30 min) and washed with de-ionized water four times to remove excessive emulsifiers. The product was freeze-dried to get fine powders.

2.2.2. Particle characterization

Particle size and size distribution were measured by laser light scattering with particle size analyzer (90 Plus, Brookhaven Instruments, Huntsville, NY, USA). For the measurement, ~2 mg nanospheres were dispersed in de-ionized water, which was filtered before use.

Dried nanospheres were coated with gold for 3 min before scanning electron microscopy (SEM, Hitachi S-4100, Japan) was applied. The accelerating voltage ranged from 5 to ~15 kV during scanning.

The nanospheres were fixed on double sided sticky tape before atomic force microscopy (AFM) was conducted. Thereafter, AFM images were obtained by Nanoscope IIIa (Digital Instrument, Santa Barbara, CA, USA) in tapping mode. The cantilever oscillated at its proper frequency (~300 KHz) and the driven amplitude was ~130 mV.

The zeta potential of the nanospheres was measured by a zeta potential analyzer (Zeta Plus, Brookhaven Instruments, Huntsville, NY, USA). First, ~3 mg nanospheres were dispersed in 10 ml buffer solution with different pH value, which was followed by sonication for 3 min. The zeta potential of products was measured with palladium electrodes, and the mean of five readings was taken.

For X-ray photoelectron spectroscopy (XPS), Axis His (a product from Kratos Analytical) with magnesium as the node was used. For all samples, the survey spectrum recorded covered a binding energy range from 0 to 1200 eV using a pass energy of 80 eV. Curve fitting was performed using the software supplied by the manufacturer.

2.2.3. In vitro release of paclitaxel

Typically, 10 mg of nanospheres were dispersed in phosphate buffered saline (PBS), the pH of which was maintained at 7.4. The buffer solution was kept in an orbital shaker that was vibrated at a constant rate of 166 rpm and the temperature was kept constant at 37.2°C. At given time intervals, three tubes of each formula of nanospheres were withdrawn and centrifuged at 12,000 rpm for 5 min. The precipitated nanospheres were taken and re-suspended in 10 ml of fresh release medium and placed back in the shaker, while the supernatant solution was kept for high performance liquid chromatography (HPLC) analysis. In order to examine the paclitaxel content in the supernatant solution, the following procedure was adopted. Paclitaxel in the release medium firstly extracted with 1 ml of DCM was reconstituted in 1 ml of mobile phase (acetonitrile:water, 1:1); DCM was then evaporated under a stream of nitrogen. For HPLC analysis, the C-18 column was used and the mobile phase was delivered at a rate of 1 ml/min. A total of 100 μl of sample was injected with an auto-sampler and the column effluent was detected at 227 nm with an ultra violet (UV) detector.

2.2.4. Encapsulation efficiency

First, ~3 mg of paclitaxel-loaded nanospheres were dissolved in 1 ml DCM and 9 ml of mobile phase subsequently were added to the solution. A nitrogen stream was introduced to evaporate DCM at room temperature until a clear solution was obtained. The resulting solution was analyzed by HPLC in the conditions mentioned above.

2.2.5. Extraction factor and recovery efficiency measurement

Due to inefficient extraction, the extraction factor had to be analyzed for calibration. The procedure was as follows [29]. Paclitaxel of known concentration in aqueous solutions (varying from 0.05 to 0.5 mg/l) was extracted and its concentration was investigated by HPLC. The results showed that the extracted solution contained 16% of the original amount of the paclitaxel. Therefore, the in vitro release result determined by this method should be corrected by 16% due to inefficient extraction.

Similarly, a known amount of paclitaxel in DCM was subjected to the procedure mentioned above. The result showed that 78% of paclitaxel remained after these procedures. Consequently, 78% was used to correct the encapsulation efficiency obtained from the method mentioned above.

3. Results and discussion

First, we outline the effects of the various emulsifiers used in the single emulsion process on the physical/chemical properties of the produced nanospheres. The influence of the traditional synthetic emulsifiers such as PVA and the natural emulsifiers such as DPPC is compared. This will then be followed by a close examination of the effects of chemical structure of the phospholipid used on the properties of the prepared nanospheres. The emphasis in the present study will be on the effects of the alkyl chain length and unsaturation of the phospholipids. We want to determine the best of the various lipids, which have the best emulsifying effects and thus result in polymeric nanospheres of the best physical and chemical properties and release profile for clinical administration of paclitaxel. The technique developed can also be applied to the general micro/nanosphere system to deliver other anticancer drugs such as adriamycin, camptothecin, fluorouracil, irinotecan and antimalarial drugs such as halofantrine and lumifantrine as well.

3.1. Effects of various emulsifiers

3.1.1. Surface chemistry

Surface chemistry of the nanospheres prepared by the solvent extraction/evaporation technique was analysed by X-ray photoelectron spectroscopy (XPS). From the chemical structures of PLGA, PVA, DPPC and paclitaxel illustrated in Fig. 1, it is apparent that paclitaxel is the only substance which contains nitrogen in the nanospheres prepared with PVA as emulsifier. Therefore, nitrogen can be the characteristic element of paclitaxel. As illustrated in Fig. 2, the scan of nitrogen failed to detect the existence of N1s (atomic orbital 1s of nitrogen) core-level signal on the exterior. This clearly indicated that there was little paclitaxel on the surface. This fact may be attributed to the very low solubility of paclitaxel in water, which makes the drug tend to stay inside polymeric nanospheres rather than diffuse to the surface.

The XPS C1s (atomic orbital 1s of carbon) regions were then studied. The results displayed in Fig. 3 show that in the analysis of the pure PLGA (75/25) and PVA, a total of four peaks were presented. Peak

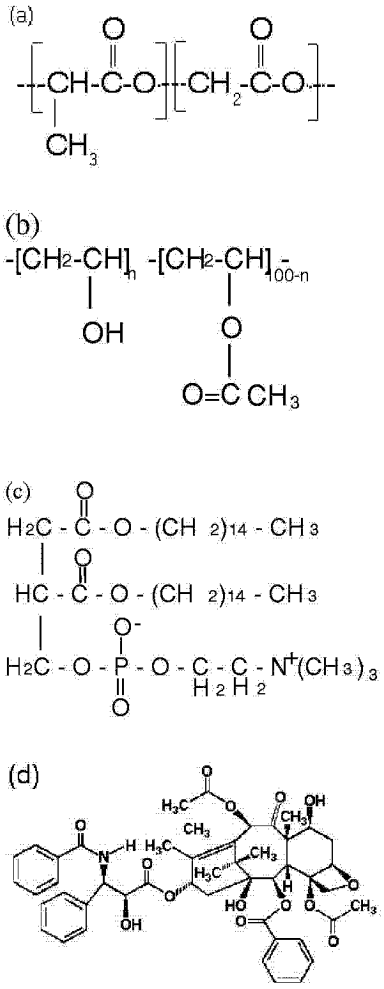

Fig. 1. Chemical structures of (a) PLGA, (b) PVA, (c) DPPC and (d) paclitaxel.

1 represents the carbon in C–C or C–H. Peak 2 is generated by the carbon next to hydroxyl (–C–OH). Peak 3 is contributed by the carbon of ester. Peak 4 corresponds to carbon in carboxylate. Since the contribution of three carbons (peaks 1, 3 and 4) is close in the molecular chain of PLGA, the percentages of these three carbons should vary little from one to another. This expectation agrees with the quantification report summarized in Table 1. However, this is not the case for pure PVA because of the different chemical structures. Compared with PLGA, there was no carbon of ester (peak 3) present in PVA; instead, peak 2 suggests the existence of

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